Isolation of the Pathogen Beauveria bassiana from Reticulitermes flavipes (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The entomogenous fungus *Beauveria bassiana* was isolated from *Reticulitermes flavipes* workers collected from a street tree in Toronto, Ontario. *R. flavipes* workers exposed to the isolate grown on water agar died within 1-3 days. In laboratory assays, active termites exposed to the sporulating fungus for 24 hours vectored *B. bassiana* into sterile agar plates, eliciting mortality in healthy *R. flavipes* workers within 12 days. Placing an excised section of the mycelial mat into vials containing healthy termites maintained in damp sand also resulted in mortality within 15 days. However, placing fungus-killed termites into similar vials containing healthy workers in sand did not result in significant termite mortality. Healthy termites appeared to avoid contact with fungus-killed cadavers.

INTRODUCTION

Isolations of fungal entomopathogens from *Reticulitermes* species (Isoptera: Rhinotermitidae) are relatively rare, despite frequent anecdotal observations of various fungal growths associated with declining laboratory colonies (cf. Beard 1974). Reported isolations include *Absidia coerulea* Bainier from *Reticulitermes flavipes* (Kollar) (Lund & Engelhardt 1962); *Antennopsis gallica* Heim & Buchli from *R. flavipes* and *Reticulitermes virginicus* Banks (Gouger & Kimbrough 1969); *Aspergillus flavus* Link. from *R. flavipes* (Beal & Kais 1962); and *Cephalosporium* sp.,

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Cunninghamella echinulata, *Gleomastix* sp., *Penicillium* sp., *P. frequentans*, *P. oxalicum*, and *Poecilomyces varioti* from *R. flavipes* (Smythe & Coppel 1966). These latter species are likely toxicant-producing or facultative pathogens (Sands 1969). Several of these same genera and species, and others detrimental to termite survival in petri dish cultures, were isolated by Zoberi & Grace (1990) from *R. flavipes* field collections and laboratory colonies.

Subsequent to our description (Zoberi & Grace 1990) of mycoflora associated with *R. flavipes* at several sites in Ontario, Canada, a small sample of *R. flavipes* collected from a Toronto street tree was brought to our laboratory by an employee of the City of Toronto Department of Parks and Recreation. This paper reports isolation of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) from these termites, and bioassays establishing pathogenicity of the isolate.

MATERIALS AND METHODS

Eastern subterranean termites, *R. flavipes*, were collected from a branch removed from a silver maple (*Acer saccharinum* L.) growing in a downtown area of Toronto, Ontario, and brought to our laboratory by an employee of the City of Toronto Department of Parks and Recreation (Urban Forestry Section). Eight termite workers were placed in a 90 X 15 mm plastic petri-dish containing 10 ml sterile water agar (Fisher Scientific). This dish was incubated in an unlighted cabinet (27±0.5°C, 90±5% RH) for 48 hours, at which time all of these termites, as well as those from the same collection stored in separate containers, were found to be dead. Dead termites were stored in plastic dishes at -10°C for further study.

A dead *R. flavipes* worker was placed on a sterile glass slide and a slide culture prepared (Zoberi 1967) and incubated in an air conditioned room at 22-24°C. The fungal mycelium rapidly radiated outward across the surface of the glass slide from the dead termite.

This fungus was isolated and grown in pure culture on several different media. The most suitable medium for optimum growth and sporulation was found to be water agar (15 g of purified grade agar mixed in 1 litre deionized water). The isolate was identified as *Beauveria bassiana* (Balsamo) Vuillemin, and depos-

ited in the Canadian Collection of Fungus Cultures, Biosystematic Research Centre, Agriculture Canada, Ottawa.

Cultures grown on water agar media and incubated at room conditions began sporulating freely after eight days. Bioassays with *R. flavipes* workers employed cultures incubated from 8–73 days. These apparently healthy termites were collected in plastic pipes containing corrugated cardboard placed on a stump and in the soil (Grace 1989) at a site in the City of Scarborough (Grace *et al.* 1989). Collected termites were maintained in plastic boxes on corrugated cardboard and cotton in an unlighted cabinet (27±0.5°C, 90±5% RH).

To establish pathogenicity of the isolate, groups of 8–18 *R. flavipes* workers were placed on sporulating petri dish water agar cultures of different ages (8–73 days). Mortality was evaluated (and dead individuals removed) daily and compared to mortality of termites placed on sterile water agar. The ability of termite workers to vector *B. bassiana* was examined by placing two live workers exposed to an active petri dish culture for a 24 hour period into a petri dish containing sterile water agar and eight healthy workers. Mortality in five such groups of 10 termites was evaluated periodically until 100% mortality was noted in all groups.

A third assay examined mortality of *R. flavipes* workers exposed to either a portion of the *B. bassiana* mycelial mat or to fungus-killed workers under conditions closer to field conditions and less favorable for fungal growth. Thirty healthy workers were placed in a 44.8 ml polystyrene vial (60 X 36 mm diameter) containing 7 ml (ca 10 g) sand, a ca 2 X 6 cm strip of Whatman No. 1 filter paper as food, and 1.5 ml deionized water. The assay consisted of four treatments, each replicated with three such vials: prior to incubation, either (1) two dead workers killed by exposure to a *B. bassiana* culture, (2) two dead workers killed by freezing(−10°C for ca 15 hours), (3) a 30 mm diameter disk cut from the mycelial mat of a sporulating *B. bassiana* culture, or (4) a similar disk cut from sterile water agar was added to each vial. The vials were then capped with polyethylene foam plugs and incubated in an unlighted chamber (27±0.5°C, 90±5% RH) for 15 days. At the end of this period, mortality and feeding (weight loss of the oven-dried papers) were evaluated (t test) (SAS 1987).

RESULTS AND DISCUSSION

To our knowledge, this represents the first record of a naturally-occurring *Beauveria bassiana* infection in *Reticulitermes*. This isolate elicited rapid mortality in *R. flavipes* workers (Table 1). Workers exposed to sporulating cultures successfully vectored *B. bassiana* into sterile agar plates, resulting in 100% mortality of termites occupying those plates within 12 days (Figure 1). This mortality can be attributed both to exposure to fungal growth arising from dissemination of propagules throughout the petri plate by the termite vectors, and to passage of propagules between termites through mutual grooming behavior. Infection of individuals via oral and anal trophallaxis (food sharing) is also possible. Kramm & West (1982) demonstrated that spores of *B. bassiana* and other entomopathogens retain their viability after ingestion by *Reticulitermes*.

Table 1. Cumulative daily percentage mortality in groups of *R. flavipes* workers in water agar petri dish cultures of the *Beauveria bassiana* isolate. Control termites were maintained on sterile agar.

Age of Culture (days)	Number of Termites	Cumulative Percent Mortality 1	2	3	4	5[1]
Control	10	0.0	0.0	10	10	10
	10	0.0	0.0	20	20	20
	10	0.0	0.0	0	0	0
8	8	25.0	87.5	100		
14	8	12.5	100			
	8	0.0	100			
	8	25.0	100			
	8	0.0	100			
17	8	100.0				
21	18	22.2	100			
22	8	12.5	100			
	8	25.0	100			
25	10	0.0	100			
51	8	100.0				
52	8	100.0				
62	10	80.0	100			
	10	40.0	100			
	10	80.0	100			
73	10	70.0	100			
	10	80.0	100			
	10	100.0				

[1]Days of exposure.

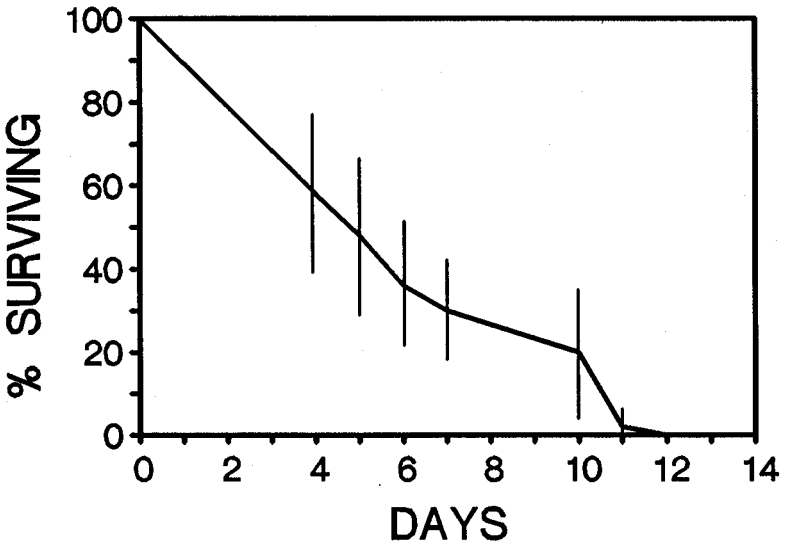

Figure 1. Mean (±SD) percent survival in five groups of 10 *R. flavipes* workers following exposure of two termites (10%) in each group to a *Beauveria bassiana* culture for 24 hours. Termites were maintained in petri dishes on water agar.

Other laboratory studies have reported *B. bassiana* to be pathogenic to *Reticulitermes* sp. (Kramm & West 1982), *R. santonensis* (Toumanoff 1965; Toumanoff & Rombaut 1965), *Coptotermes formosanus* (Lai *et al.* 1977), and other soil-dwelling insects (Klein 1988), but attempts to infect termites under field conditions have been less successful (Lai 1977). Similarly, our exposure of *R. flavipes* workers maintained in the more natural damp sand substrate (rather than an agar plate) to termites killed by *B. bassiana* did not elicit significant mortality (Table 2). Although conditions for fungal growth are certainly less ideal in a natural substrate than on agar, termite behavior also appears important in limiting infection. On agar plates, we observed that fungus-killed workers were avoided by the living termites and frequently appeared to have been buried in the agar. Avoidance of dead individuals was also noted by Kramm *et al.* (1982) to limit transfer of *Metarhizium anisopliae* to healthy *Reticulitermes* workers, leading to the conclusion that active termites are more effective in transmitting pathogens.

Table 2. Mean (±SD) percentage mortality in groups (n=3) of 30 *R. flavipes* workers maintained in damp sand 15 days after exposure to a portion of the *Beauveria bassiana* mycelial mat or to two termites killed by *B. bassiana.*

Treatment	Percent Mortality Treatment	Control[2]	*t* test probability
Agar disk cut from mycelial mat	100±0	17.78±3.85	<0.001
Two fungus-killed termite workers	33.33±8.82	21.11±6.94	0.140

[2]Control groups were exposed to either a 30 mm diameter disk of sterile agar, or to two termite workers killed by freezing.

Although termites may isolate fungus-killed individuals, *B. bassiana* mycelia and spores are not avoided, suggesting potential use of this pathogen in baiting systems for termite control. Although a passive system of infection whereby foraging termites are infected by ingestion of, or contact with, fungal propagules placed in bait stations would probably be ideal, it might also be feasible (although more labor intensive) to collect foragers in such bait stations, then contaminate them by exposure to sporulating cultures or "dusting" with propagules prior to release back into the colony. Similar application methods were suggested by Grace & Abdallay (1990) with boron dusts, and described by French (1990) with arsenic trioxide powder.

Repeated collections, infection, and release of large numbers of termite foragers from multiple traps at a given site might prevent local accumulation of dead termites and subsequent avoidance of the area and pathogen by healthy foragers.

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REFERENCES

Beal, R.H., & A.G. Kais. 1962. Apparent infection of subterranean termites with *Aspergillus flavus* Link. J. Insect Pathol. 4: 488-489.

Beard, R.L. 1974. Termite biology & bait block method of control. Conn. Agric. Exper. Sta. Bull. No. 748. 19 pp.

French, J.R.J. 1990. Baiting techniques in the control of *Coptotermes* species within existing buildings in Australia. Proceedings of a Symposium on Current Research on Wood Destroying Organisms and Future Prospects for Protecting Wood-in-Use (M.I. Haverty & W.W. Wilcox, eds.). *in press.*

Gouger, R.J., & J.W. Kimbrough. 1969. *Antennopsis gallica* Heim and Buchli (Hyphomycetes: Gloeohaustoriales), an entomogenous fungus on subterranean termites in Florida. J. Invert. Pathol. 13: 223-228.

Grace, J. K. 1989. A modified trap technique for monitoring *Reticulitermes* subterranean termite populations (Isoptera: Rhinotermitidae). Pan-Pac. Entomol. 65: 381-384.

Grace, J.K., & A. Abdallay. 1990. Termiticidal activity of boron dusts (Isoptera: Rhinotermitidae). J. Appl. Entomol. *in press.*

Grace, J. K., A. Abdallay, & K.R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Can. Entomol. 121: 551-556.

Klein, M.G. 1988. Pest management of soil-inhabiting insects with microorganisms. Agric. Ecosys. Environ. 24: 337-349.

Kramm, K.R., & D.F. West. 1982. Termite pathogens: Effects of ingested *Metarhizium, Beauveria,* and *Gliocladium conidia* on worker termites (*Reticulitermes* sp.). J. Invert. Pathol. 40: 7-11.

Kramm, K.R., D.F. West, & P.G. Rockenbach. 1982. Termite pathogens: Transfer of the entomopathogen *Metarhizium anisopliae* between *Reticulitermes* sp. termites. J. Invert. Pathol. 40: 1-6.

Lai, P.Y. 1977. Biology and ecology of the Formosan subterranean termite, *Coptotermes formosanus,* and its susceptibility to the entomogenous fungi, *Beauveria bassiana* and *Metarrhizium anisopliae.* Ph.D. diss., Univ. Hawaii, Honolulu.

Lai, P.Y., M. Tamashiro, & J.K. Fujii. 1982. Pathogenicity of six strains of entomogenous fungi to *Coptotermes formosanus.* J. Invert. Pathol. 39: 1-5.

Lund, A.E., & N.T. Engelhardt. 1962. Subterranean termites and *Absidia coerulea* Bain. (Mucorales). J. Insect Pathol. 4: 131-132.

Sands, W.A. 1969. The association of termites and fungi. Pp. 495-524 in Biology of Termites, Vol. 1 (K. Krishna and F.M. Weesner, eds.).

Academic Press: New York.
SAS Institute Inc. 1987. SAS/STAT Guide for Personal Computers, Version 6 Ed. SAS Institute Inc.: Cary, NC.
Smythe, R.V., & H.C. Coppel. 1966. Pathogenicity of externally occurring fungi to *Reticulitermes flavipes*. J. Invert. Pathol. 8: 266-267.
Toumanoff, C. 1965. Action de divers champignons entomophages sur *Reticulitermes santonensis*. Ann. Parasitol. 40: 611-626.
Toumanoff, C., & J. Rombaut. 1965. Actions de certains champignons entomophage cultives sur les milieux appropries attractifs, sur le termite de Saintonge *Reticulitermes santonensis*. Ann. Parasitol. 40: 605-609.
Zoberi, M.H. 1967. A simple technique for preparing and preserving slide cultures. Trans. Brit. Mycol. Soc. 50: 157-158.
Zoberi, M.H., & J.K. Grace. 1990. Fungi associated with the termite *Reticulitermes flavipes* (Kollar) in Ontario. Mycologia *in press*.